Ku protein targeting by Ku70 small interfering RNA enhances human cancer cell response to topoisomerase II inhibitor and γ radiation

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Abstract
Ku protein is a heterodimer (Ku70 and Ku86) known to play an important role in V(D)J recombination, apoptosis, telomere fusion, and double-strand break repair. Its role in double-strand breaks is relevant to cancer therapy because lack of Ku86 causes one of the most radiation-responsive phenotypes (hamster cells, XRS5). Although it is known that the heterodimer is necessary for the various functions of this protein, the impact of targeting Ku in human cancer cells has not been shown due to lack of appropriate approaches. It is also not known whether complete knock-out of Ku protein is required to enhance the sensitivity of human cells to γ radiation as Ku protein is much more abundant in human cells than in hamster cells. In the current article, we have investigated the direct effect of Ku70 depletion in human cervical epithelioid (HeLa) and colon carcinoma (HCT116) cells. We specifically targeted Ku70 mRNA by use of small interfering RNA (siRNA). Of the five Ku70 siRNA synthesized, three inhibited the expression of Ku70 by up to 70% in HeLa cells. We have tested the effect of chemically synthesized siRNAs for target sequence 5 (CS #5) on the response of HeLa cells 72 hours after transfection to γ radiation and etoposide, as this showed the maximum inhibition of Ku70 expression. Ku70 siRNA induced a decrease in the surviving fraction of irradiated HeLa cells by severalfold. Similar sensitizing effects were observed for etoposide, a topoisomerase II inhibitor. Studies with HCT116 cells using the same Ku70 siRNA (CS #5) showed a direct correlation between expression of Ku70 and sensitization to radiation and etoposide treatments. [Mol Cancer Ther 2005;4(4):529–36]

Introduction
It has been a long-standing interest to target proteins responsible for the resistance of cancer cells to γ radiation and chemotherapeutic agents (1–4). Most recent molecular biological studies have focused on the signaling transduction mechanisms responsible for cellular proliferation and growth (4–7). It is quite possible that targeting signaling pathways mediated by phosphorylation, kinases, growth receptors, and oncogenes may yield clinically relevant approaches to specifically kill cancer cells; provided that only cancer cells and not the normal cells rely on these pathways for survival. There are numerous preclinical evidences that these inhibitors can potentiate the antitumor effects of many cytotoxic agents (8). However, the efficacy of these inhibitors to enhance the effects of cytotoxic agents in clinical studies in humans is not clearly established (8).

Baker et al. (9) have shown that overexpression of thioredoxin, which plays some role in the growth of several cancer cells, is effective in reducing the sensitivity of mammalian cells to certain chemotherapeutic agents. However, we have recently shown that inhibition of the oxidative pentose phosphate cycle, which supplies NADPH required for the function of thioredoxin and other proteins, did not sensitize the noncancerous Chinese hamster ovary cells to etoposide,³ and only slightly sensitized these cells to γ radiation (10). These results have indicated that oxidative pentose phosphate cycle–mediated redox signaling might play only a minor role in cellular response to DNA-damaging agents. The most relevant downstream signaling pathways that could play a significant role in sensitizing the cancer cells to DNA-damaging agents are those involved in apoptosis (11–13). We have previously shown that DNA is an important target in radiation-induced apoptosis, suggesting that both mitotic and apoptotic cell death are caused by DNA lesions after irradiation (14).

Ku protein plays a major role in the repair of DNA double-strand breaks caused by γ radiation and some chemotherapeutic agents (15–19). A key system for the repair of DNA double-strand breaks is nonhomologous end-joining (20, 21). The role of Ku is to bind to DNA ends, thus facilitating the coordination of other DNA repair proteins (20, 21). Biochemical and genetic studies using mutant rodent cell lines sensitive to ionizing radiation have identified at least four genes, XRCC4, XRCC5, XRCC6, and XRCC7, that are required for nonhomologous end-joining (20, 21). XRCC4 encodes a 38 kDa nuclear phosphoprotein

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3 J.E. Biaglow et al., personal communication.
that binds strongly to DNA-ligase IV (21). XRCC7 encodes a 460 kDa catalytic subunit (DNA-PKcs) of DNA-PK (20, 21). XRCC5 and XRCC6 encode 86 and 70 kDa subunits, respectively, of Ku autoantigen, a DNA end-binding protein and regulatory subunit of DNA-PK (20, 21). The protein kinase activity of DNA-PK is believed to be stimulated by its association with DNA-end-bound Ku (20, 21). Ku heterodimer binds to the ends of DNA in a non–sequence-dependent manner (20, 21). The DNA-binding activity of Ku requires reduced sulfhydryl groups in cell-free systems (22). Ku heterodimer contains 14 cysteine residues and 5 of these are located in Ku70, which is in contact with the DNA binding site (22). In a recent report, we have shown that Ku protein function can be greatly decreased in glucose-6-phosphate dehydrogenase–deficient Chinese hamster ovary cells by Ku protein thiol oxidation (23). However, this biochemical approach is effective only in glucose-6-phosphate dehydrogenase–deficient cells.

In this study, we used a novel molecular approach to specifically target Ku70 mRNA. We show that this approach inhibits the expression of Ku70 protein and causes an increased response of cancer cells to γ radiation and topoisomerase II inhibitor.

**Materials and Methods**

**Cells and Growth Medium**

HeLa and HCT116 cancer cells were obtained from the American Type Culture Collection (Manassas, VA). These cells were grown in McCoy 5A medium with 10% FCS and 20 mmol/L HEPES at 37°C in a humidified 5% CO₂ incubator.

**Ku70 Small Interfering RNA Synthesis**

In vitro – Transcribed siRNA. The nucleotide sequence of human Ku gene (accession #BC010034) was retrieved from the National Center for Biotechnology Information. Twenty-one-mer nucleotides with starting amino acid nucleotides for several target sequences were selected using Ambion software. To produce in vitro – transcribed small interfering RNA (siRNA) against Ku70, we used the pSilencer siRNA construction kit according to the manufacturer’s instructions (Ambion, Austin, TX). The sense and antisense for two target sequences Ku70-3 and Ku70-4 for in vitro – transcribed siRNA are shown below:

**Ku70-3**

**Sense** 5’-AATTCAGGTGACTCTCCAGG CCTGTCCTC-3’

**Antisense** 5’-AACCTGGAGGACTCACCTGAA CCTGTCCTC-3’

**Ku70-4**

**Sense** 5’-AAGGGAAGTCTACACAGAGAA CCTGTCCTC-3’

**Antisense** 5’-AATTCCTTGTTGAACCTTCCC CCTGTCCTC-3’

These oligonucleotides were hybridized to the T7 promoter primer supplied in the manufacturer’s kit and used as directed.

**Chemically Synthesized siRNA.** We have also made siRNAs by chemical synthesis at Ambion’s siRNA oligonucleotide synthesis facility. We used the following sense and antisense oligonucleotides for target sequences Ku70-3, Ku70-4, and Ku70-5 to produce chemically synthesized siRNA against Ku70.

(a) the siRNA for Ku70-3 consists of hybridized sense 5’-UUCAGGUAGCUCCUCCAGTTT-3’ and antisense 5’-CCUGGAGGAUCACCUAGATTT-3’ oligonucleotides.

(b) the siRNA for Ku70-4 consists of hybridized sense 5’-UUCUCUGGUAACUUCCTT-3’ and antisense 5’-GGGAAAGUACAGAGAATTT-3’ oligonucleotides.

(c) the siRNA for Ku70-5 consists of hybridized sense 5’-GAUCCUUCUACUGAAAAATTT-3’ and antisense 5’-UUUUCAGUAAGGGCAUCCTT-3’ oligonucleotides.

**RNase III Generated.** We have also generated siRNAs by enzymatic digestion of long in vitro – transcribed double-stranded RNA molecule by the bacterial enzyme, RNase III. The enzymatic digestion produces double-stranded siRNAs corresponding to several different target sequences. A mixture of several different siRNAs targeted to different target sequences in a gene is expected to be effective in most cells. To produce the Ku70 long double-stranded RNA, we first amplified an ~200 bp region of Ku70 using the primers 5’-AATTTAATAGCAGCTCATATGCC-TATGCTACCGAGAAAGACA-3’ and 5’-AATTTAATAC-GACTCATATAGCTAAAAAGGTTGCCACAGAC-3’ with complementary DNA derived from HeLa cells. The 200 bp PCR product was run on a 1% agarose gel and purified. The long double-stranded RNA and the siRNA were then produced according to the instructions in the siRNA cocktail kit (Ambion).

**Ku70 siRNA Transfection**

Forty thousand HeLa or HCT116 cells in a total volume of 370 μL McCoy 5A medium were plated in 24-well plates and incubated for 24 hours. The McCoy 5A medium was aspirated and cells were rinsed with Earle’s balanced salt solution with 20 mmol/L HEPES. The cells were replenished with 200 μL of fresh DMEM and incubated for 1 hour at 37°C in a humidified 5% CO₂ incubator. Cells were treated with Ku70 siRNA or scrambled siRNA as negative control according to the manufacturer’s instructions. Briefly, siRNA and OligofectAMINE were mixed separately with OptiMem and incubated for 15 minutes at room temperature. These reagents were combined and incubated for another 15 minutes before adding to the cells in DMEM without penicillin and streptomycin. The effect of Ku70 siRNA on Ku70 protein was determined by Western blot at 24, 48, and 72 hours after transfection. The effects of γ radiation and topoisomerase II poisons on HeLa cells were estimated 72 hours after Ku70 siRNA transfection because...
72 hours' incubation with siRNA showed the maximum inhibition of Ku70 expression. The effects of γ radiation and topoisomerase II poisons on HCT116 cells were estimated 24, 48, and 72 hours after Ku70 siRNA transfection to determine whether a direct correlation exists between Ku70 expression and sensitization to radiation and etoposide.

**Western Blot Analysis**

The total amount of cellular Ku70 protein was quantified using Western blot and NIH image analysis. HeLa and HCT116 cancer cells treated with and without Ku70 siRNA were rinsed with PBS thrice and mixed with 200 μL of lysis buffer [20 mmol/L Tris (pH 7.6), 150 mmol/L NaCl, 2 mmol/L EDTA, 10% glycerol, 1% Triton X-100, 1 mmol/L DTT, 1 mmol/L orthovanadate, 2 mmol/L phenylmethyl-sulfonyl fluoride] containing 6 μL of protease inhibitor cocktail (Sigma, St. Louis, MO) and 6 μL of NuPAGE reducing agent. The cells in lysis buffer in the dish were removed using a Teflon scraper and transferred to an Eppendorf tube using a micropipette. The cells were homogenized using a 1 mL syringe (22-gauge needle), centrifuged at 6,000 × g for 2 minutes in a microfuge (Fisher 59A), and the supernatant stored at −80°C. The protein extracts (10 μg) were incubated in NuPAGE sample buffer containing 4 μL of NuPAGE reducing agent at 70°C for 10 minutes. These samples were then electrophoresed on a 10% Bis/Tris precast gel from NuPAGE at 200 V for 60 minutes in MOPS SDS running buffer (NuPAGE). The proteins were transferred to nitrocellulose by electrophoresis in NuPAGE transfer buffer at 20 V for 60 minutes. The nitrocellulose blot was incubated with 10 mL of blocking buffer for 1.5 hours at room temperature on a rocker and stored in the cold room overnight. The nitrocellulose paper was washed five times with PBS containing 0.1% Tween 20 (PBST) and then incubated with 20 μL of primary Ku (p70) antibody (Ab-4, clone N3H10, Neomarkers, Fremont, CA) in 10 mL of blocking buffer for 2 hours at room temperature. The nitrocellulose paper was washed five times with PBST before incubation with secondary peroxidase-labeled mouse antibody for 1 hour, as per the manufacturer's instruction (Amersham, Piscataway, NJ), and the bands were detected using a standard enhanced chemiluminescence kit. The density of bands in the resulting film was quantified using NIH image analysis.

**Immunofluorescence and Imaging**

Immunofluorescence analysis was done as follows: first, at the indicated time following transfection in a 24-well tissue culture dish, the cells were fixed by 5-minute incubation in fresh 4% paraformaldehyde/PBS at room temperature. The cells were washed with 1 mL of 1× PBS, permeabilized by the addition of 0.1% Triton X-100/PBS and incubated for 5 minutes at room temperature. The cells were then washed with 1× PBS, and blocked in 3% BSA/PBS for 1 hour and washed with 1× PBS. Primary Ku70 antibody (Ambion, catalog #4320) was diluted 1:200 in PBS, mixed with the cells, and incubated for 1 hour at room temperature. The primary antibody was then removed and the cells were washed with 1 mL of PBS, and incubated for 1 hour with secondary antibody (FITC-conjugated Affinity-Pure donkey anti-mouse IgG, catalog #715-095-150, Jackson ImmunoResearch, West Grove, PA) followed by washing with PBS and mounted using VectaShield with 4,6-diamidino-2-phenylindole (Vector Labs, H1200). The cells were visualized using an Olympus BX60 microscope and quantified using Metamorph.

**Radiation or Etoposide Treatment**

The cells in 24-well plates were irradiated at room temperature in air using a Shepherd Mark I 137Cs irradiator. Radiation doses were as indicated in the figures. Alternatively, they were incubated with different concentrations of etoposide (Sigma) for 1 hour at 37°C in a humidified 5% CO2 incubator.

**Clonogenic Assay**

The cells in 24-well plates were rinsed twice with 0.3 mL of PBS and trypsinized with 0.1 mL of trypsin. The trypsinized cells were resuspended in 0.2 to 0.3 mL of medium and 0.1 mL was transferred to 12 mL of medium and isotonic solution for plating and cell counting (Coulter Counter), respectively. The cells were plated in 100 mm dishes at the required concentration to get no more than 250 colonies per dish. The cells were cultured for 8 to 10 days in 5% CO2 to get viable colonies. A viable colony was defined as having at least 50 cells after 10 days of growth. The surviving fraction of treated cells was normalized to the plating efficiency of control (untreated) cells.

**Results**

To determine the effects of various siRNAs on Ku protein expression, we measured the amount of Ku70 by Western blot analysis using a monoclonal anti-Ku70 antibody. A very slight change in the single ~70 kDa immunoreactive band was observed in total cell lysates extracted from HeLa cell lines treated for 24 hours with target sequences 4 (lanes 4 and 6) and 5 (lane 7) and RNase III generated (lane 8) Ku70 siRNAs (Fig. 1). The Ku protein level was further decreased after 48 and 72 hours' incubation with siRNA with the highest decrease observed after 72 hours. In addition, in vitro–transcribed siRNA for target sequence 3 (lane 3) also showed a significant decrease when cells were treated with siRNA for 48 and 72 hours. This indicates that 48 to 72 hours are required for the maximum inhibition of Ku70 protein. The scrambled siRNA treated HeLa cells (lane 2) had no measurable effect on the levels of Ku70. This siRNA was used as a negative control for experiments that determined the sensitizing effects of Ku70 siRNA. Of the five Ku70 siRNAs, target sequences 4 (lanes 4 and 6) and 5 (lane 7) are the most effective in suppressing the expression of Ku70. The RNase III generated Ku70 siRNA (lane 8) inhibited the Ku70 expression less effectively than the Ku70 siRNA generated for target sequences 3, 4, and 5.

We have also determined the expression of Ku70 protein by immunohistochemistry in cells transfected with chemically synthesized Ku70 siRNA for target sequence 5 because Western blot analysis indicated that this siRNA is
The most effective at 72 hours after transfection (Fig. 1B). We have normalized the Ku70 immunohistochemical staining with 4',6-diamidino-2-phenylindole staining, and the amount of fluorescent signal from the nontransfected control (Fig. 2A). The relative fluorescent signal per cell was quantified using Metamorph and graphed (Fig. 2B). Ku70 siRNA for target sequence 5 has reduced the expression of Ku70 in HeLa cells to 20%, 40%, and 70% at 24, 48, and 72 hours after transfection.

Of the five Ku70 siRNAs, we have tested the effect of chemically synthesized siRNAs for target sequence 5 on the γ radiation response because it had shown the maximum inhibition of Ku70 expression at 72 hours after transfection (Fig. 1B). Ku70 siRNA for target sequence 5 has reduced the expression of Ku70 in HeLa cells to 20%, 40%, and 70% at 24, 48, and 72 hours after transfection.

Figure 1. Effects of various siRNAs on Ku70 expression as measured by Western blot analysis using a monoclonal anti-Ku70 antibody in HeLa cells. A, Western blot of cellular protein extracts of HeLa cells at 24, 48, and 72 h after transfection with Ku70 siRNA. Control untreated (lane 1), negative control siRNA (lane 2), in vitro–transcribed Ku70 siRNA for target sequence #3 (lane 3), in vitro–transcribed Ku70 siRNA for target sequence #4 (lane 4), chemically synthesized Ku70 siRNA for target sequence #3 (lane 5), chemically synthesized Ku70 siRNA for target sequence #4 (lane 6), chemically synthesized Ku70 siRNA for target sequence #5 (lane 7), and RNase III generated Ku70 siRNA (lane 8). B, graph of the effects of siRNA on total Ku protein quantitated by NIH image analysis plot of each lane in the gel and presented as percentage of untreated control. Each experiment was repeated at least thrice with SD as shown unless smaller than points plotted. The loading control actin indicated no significant difference in loading.

Figure 2. Immunofluorescence image analysis of HeLa cells transfected with chemically synthesized Ku70 siRNA for target sequence 5. A, Ku70 expression in HeLa cells at 24, 48, and 72 h after transfection with Ku70 siRNA. The cells were visualized using an Olympus BX60 microscope. B, graph of the effects of Ku70 siRNA (relative fluorescent per cell compared with a nontransfected sample) on total Ku protein quantitated by Metamorph analysis.
To determine the generality of this Ku70 siRNA (CS #5), we have also tested the effects of this siRNA on colon carcinoma cells HCT116. The Western blot analysis, using the same monoclonal anti-Ku70 antibody used for HeLa cells, showed 16% inhibition in Ku70 in total cell lysates extracted from HCT116 treated for 24 hours (Fig. 4, lane 3). The Ku protein level was further decreased to 70% at 48 hours after transfection with Ku70 siRNA (Fig. 4, lane 6). However, the cells recovered completely at 72 hours (Fig. 4, lane 9) after transfection, suggesting that the maximum inhibitory effect of siRNA was observed at 48 hours after transfection (i.e., somewhat earlier than the HeLa cells).

In order to determine the correlation between Ku70 expression and radiation response, we have exposed the HCT116 cells transfected with Ku70 siRNA (CS #5) to radiation (Fig. 5A–C). HCT116 transfected for 48 hours showed significant sensitization to radiation (Fig. 5B). However, cells transfected for 24 and 72 hours did not show any significant radiation sensitization (Fig. 5A and C). Thus, the radiation response paralleled the change in Ku70 expression.

Another extensively studied DNA-damaging agent is etoposide. This drug causes DNA damage by inhibiting topoisomerase II resulting in DNA double-strand breaks (15, 24). Therefore, we measured the etoposide dose–response curves for HeLa cell transfectants. We have again selected to test the effect of chemically synthesized siRNAs for target sequence 5 on the radiation response because it had shown 70% inhibition of Ku70 expression at 72 h after transfection. Each experiment was repeated at least thrice with SD as shown unless smaller than points plotted.

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Treatment of the HCT116 cells with etoposide also resulted in a decrease in cell survival (Fig. 7A–C). However, the Ku70 siRNA-transfected HCT116 cells were much more sensitive to etoposide at 48 hours after transfection than the nontransfectant (Fig. 7B). Similar to the response of transfectants to radiation, HCT116 transfected with Ku70 siRNA for 24 and 72 hours did not show any significant sensitization or inhibition of Ku expression; suggesting a direct correlation between Ku70 inhibition and etoposide response (Fig. 7A–C).

Discussion

Repair proteins have been shown to play a major role in the response of cells to DNA damaging agents (24, 25).
However, because of the tremendous variety of damage types and the complexity, and number of repair proteins and their complexes, it has often been difficult to pinpoint the precise function and damage specificity of each protein. This is particularly so because several of the repair mutants were isolated functionally before the advent of modern molecular techniques. To help with this problem, various mutants have now been characterized. They often seem to lack the function of only one repair protein. It has been shown that Ku heterodimer is involved in the repair of DNA double-strand breaks via nonhomologous end-joining (26–30).

Radiation produces free radicals by random radiolysis of water. Radiation is known to induce cell death primarily by the action of groups of hydroxyl radicals producing DNA double-strand breaks. Nonhomologous end-joining plays a major role in the repair of DNA double-strand breaks induced by γ radiation (20, 21). Studies with mutant rodent cells with deficiency in DNA-PKcs, Ku86, and DNA ligase IV have shown that these proteins are essential for the nonhomologous end-joining repair pathway and survival after irradiation (20, 21). Of these three major DNA repair proteins, Ku plays a central role by sensing DNA lesions and coordinating various DNA repair proteins (15, 17, 18).

Topoisomerase II poisons also induce DNA double-strand breaks leading to cell death similar to γ radiation. Although radiation directly induces double-strand breaks, topoisomerase II poisons induce double-strand breaks by stabilizing the covalent intermediate (DNA/topoisomerase II complex) of the topoisomerase II reaction (15, 17, 31). Nonhomologous end-joining is likely to be the major pathway involved in the repair of DNA double-strand breaks produced by topoisomerase II poisons (15, 17, 32). The role of nonhomologous end-joining repair protein Ku in cellular response to topoisomerase II poison has been shown using DNA double-strand break repair–deficient XRS strains (15, 17, 32). However, a recent report showed that the hypersensitivity is observed only in Ku and not DNA-PKcs-deficient cells (33). Thus, the work presented here is consistent with the dominant effect of Ku70 depletion inducing sensitivity to both ionizing radiation and etoposide.

For mammalian cells, lack of Ku-86 was shown to cause severe radiation sensitivity in the XRS5 hamster line and this mutant has been characterized with many other DNA

Figure 5. Effects of γ radiation, in the presence of air, on the clonogenic survival of colon carcinoma cells HCT116 at 24, 48, and 72 h after Ku70 siRNA transfection. We have tested the effect of chemically synthesized siRNAs for target sequence 5 on the γ radiation response. Each experiment was repeated at least thrice with SD as shown unless smaller than points plotted.

Figure 6. Effects of etoposide, in the presence of air, on the clonogenic survival of HeLa cells 72 h after Ku70 siRNA transfection. Of the five Ku70 siRNAs synthesized, we have tested the effect of chemically synthesized siRNAs for target sequence 5 on the etoposide response because it had shown 70% inhibition of Ku70 expression at 72 h after transfection. Each experiment was repeated at least thrice with SD as shown unless smaller than points plotted.
damages, including sensitivity to topoisomerase II poisons (15, 17, 18, 32). Most investigators have focused on DNA repair–deficient mutants to study the mechanisms of DNA repair (16, 28–32) with only a few examples exploiting molecular targeting of repair proteins to increase the response of cancer cells to DNA damaging agents (34). The recent discovery that small interfering RNAs are effective in silencing gene expression in mammalian cells has opened up new avenues to silence repair protein expression (35, 36). This is important for many reasons. For example, even though Ku exists as a functional heterodimer, Ku70 mutants have not been identified. Furthermore, there is enormous difference in the cellular concentration of repair proteins, with human cells typically having more Ku and DNA-PK than hamster cells. It is not known why this should be so, particularly because human cells are commonly more sensitive than their hamster counterparts. Our results have now shown that Ku70 siRNA can be used to inhibit Ku protein synthesis in human cancer cells and that this causes sensitization to ionizing radiation and etoposide. This result is perhaps unexpected, because even at the maximum inhibition of Ku70 reported here (~70% depletion), the cells contain much more Ku than normal hamster cells.

There was considerable variation in the efficiency of the various siRNAs's made. Only three of the five Ku70 siRNAs were highly effective in silencing the Ku protein expression. The RNase III generated Ku70 siRNAs also decreased the Ku protein but at a much lower efficiency. It will obviously be of interest to continue the search for more efficient Ku70 depletion, and we are currently working to make a stable siRNA. The efficiency of Ku70 depletion correlated exactly with changes in radiation and etoposide sensitivity, both quantitatively and qualitatively. Our results suggest that Ku70 must be depleted by at least 70% to significantly change DNA damage response.

We have recently shown that γ radiation-induced cell death could be enhanced by hydroxyethyl disulfide, a thiol oxidant, in glucose-6-phosphate dehydrogenase–deficient Chinese hamster ovary mutant cells (10). This modification in radiation response correlated well with defects in DNA double-strand break repair and loss of Ku binding to DNA ends (10, 14). Two-log higher cell killing and 70% inhibition of DNA double-strand break repair and Ku binding in glucose-6-phosphate dehydrogenase mutant Chinese hamster ovary cells suggested that inhibition of Ku protein function will be highly effective in sensitizing the cancer cells to γ radiation and other DNA-damaging chemotherapy agents (10, 14). However, hydroxyethyl disulfide–mediated loss of Ku function resulting from protein thiol modification is effective only in glucose-6-phosphate dehydrogenase–deficient cells (10, 14). Our results have shown, for the first time, that transfection of HeLa and HCT116 cancer cells with Ku70 siRNA significantly enhanced the response to γ radiation and the chemotherapeutic agent etoposide.

These current results suggest that targeting of Ku protein by Ku70 siRNA is a possible approach to cancer therapy. It has also raised possibilities to target other DNA repair proteins either alone or in combination with Ku70 siRNA to increase the response of cancer cells to DNA-damaging agents (36).
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References

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